REMARKS

In response to the Office Action dated June 8, 2004, Applicant hereby elects group I drawn to claims 1-34, a method of preparing a collagen sponge. The non-elected and now withdrawn claims remain in the application, and Applicant reserves the right to file divisional applications directed to such claims.

The above amendments have been made to correct a number of typographical errors in the original specification.

More specifically, the original specification has now been corrected to refer to the elasticity module as N/cm^2. It was incorrectly written in the original specification as N/cm. It is respectfully submitted that this is an obvious typographical error and that the change does not represent new matter.

More specifically, the elasticity module is a parameter that is well known in the art to measure the elasticity of a material. See for example the enclosed pages 38-43 from the book "The New Science of Strong Materials or Why You Don't Fall Through the Floor" by J. E. Gordon. Note the table on page 42 of Gordon. Further see EP 1053757, referring to page 3, lines 30-32 and claim 5, using the module of "N/mm^2" (a translation has not been provided, but can be provided upon request). Further, additional examples of how an elasticity module or Young's modulus is calculated can also be provided. From these it becomes clear that one of ordinary skill in the art would recognize that the expression N/cm in the specification was a typographical error, and that it should have been "N/cm^2".

In view of the above, entry of the above amendments is respectfully submitted to be in order, and such entry is requested.

Respectfully submitted,

Alfred SCHAUFLER

By:
Nils E. Pedersen
Registration No. 33,145
Attorney for Applicant

NEP/krg
Washington, D.C. 20006-1021
Telephone (202) 721-8200
Facsimile (202) 721-8250
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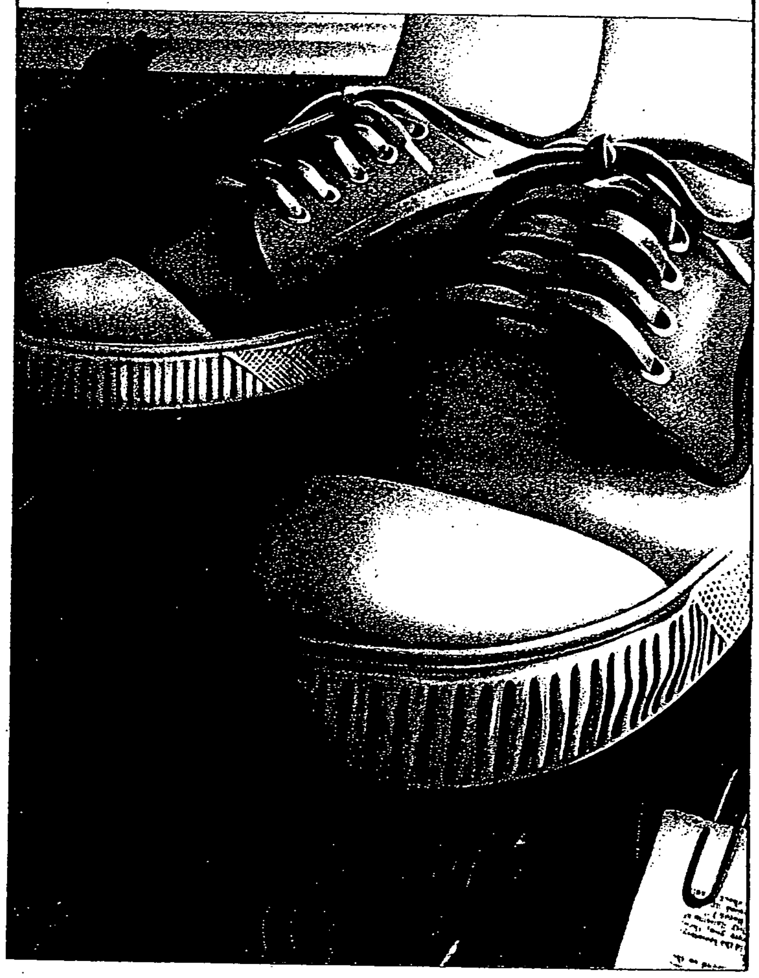
J. E. Gordon

The New Science of Strong Materials

or Why You Don't Fall Through the Floor



In Hooke's day, and indeed down to the last few years, materials either broke or else flowed and ceased to be elastic when strains much over 1·0 per cent were applied to them. So the shape of the interatomic force curve at large deflections was only of the

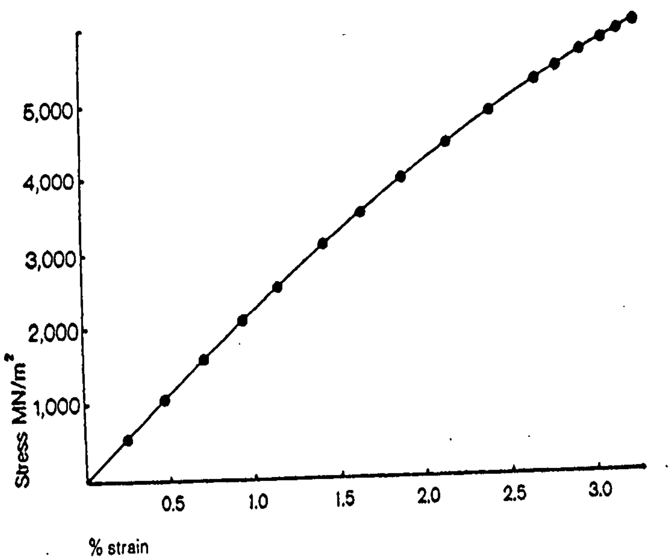


Figure 4. Stress-strain relationship for a very strong silicon whisker. This whisker or needle-like crystal was strained to 3·6 per cent in a testing machine and although the behaviour is 'elastic' it does not obey Hooke's law at the higher strains, the top of the graph being distinctly curved. This is because the interatomic force relationship is also curved at the higher strains. Other strong filaments, such as iron whiskers, have similar non-linear stress-strain curves at high stresses.

most academic interest because such stresses were never reached. Fairly recently, in the writer's laboratory and elsewhere, it has been possible to take very strong 'whisker' crystals up to strains between 3 and 6 per cent and the measurements confirm that Hooke's law is not literally true. The stress-strain curve bends over to follow the interatomic force curve which is derived from considerations, not of engineering, but of theoretical physics.

Figure 4 shows such a curve for a silicon whisker strained to over 3 per cent.

Young's modulus

Hooke stated that the deflections of springs and other elastic bodies were proportionate to the load which is applied to them but, of course, with different structures, the actual deflection under any given load will depend both upon the geometrical size and shape of the structure and also upon the material from which it is made. It is not clear how far Hooke distinguished elasticity as a property of a *material* from elasticity as a function of the *shape and dimensions* of the structure. We can get similar load-extension curves from a straight piece of rubber and from a helical piece of steel which we call a spring – this has always been a fruitful source of confusion. Certainly for something like a century after Hooke's time a state of intellectual muddle seems to have invested the few people who thought about elasticity and no clear distinction seems to have been made between these ideas.

Around 1800 Thomas Young (1773–1829) realized that, if we consider the stresses and strains in the material rather than the gross deflections of the structure, then Hooke's law can be written:

$$\frac{\text{stress}}{\text{strain}} = \frac{s}{e} = \text{constant}$$

Furthermore, Young realized that there was here a constant peculiarly characteristic of each chemical substance which, as he might have said, represents its 'springiness'. We call this constant 'Young's modulus' or E. There is no mystery about the word 'modulus', it just means a figure which describes a property of a material. Thus:

$$E = \frac{s}{e} = \frac{\text{stress}}{\text{strain}}$$

E therefore describes the elastic flexibility of a material as such; the flexibility of any given object will thus depend both upon the Young's modulus of the material from which it is made and also upon its geometrical shape.

It is said of Young that he was 'a man of great learning but unfortunately he never even began to realize the limitations of comprehension of ordinary minds'.* Young published the idea of his modulus in a rather incomprehensible paper in 1807 after he had been dismissed from his lectureship at the Royal Institution for not being sufficiently practical. Thus perhaps the most famous and the most useful of all concepts in engineering, which defines the stiffness or floppiness of a material, was not generally understood or absorbed into engineering practice until after Young's death. Young's modulus is often called 'stiffness' in casual engineering conversation and will sometimes be called stiffness and sometimes E in this book.

E is enormously important in engineering for two reasons. First, we need to know with accuracy the deflections in a structure, as a whole and in its various parts, when it is loaded. A moment's thought about bridges or aeroplanes or crankshafts will show that this is so (Figure 5). Things must still fit together,

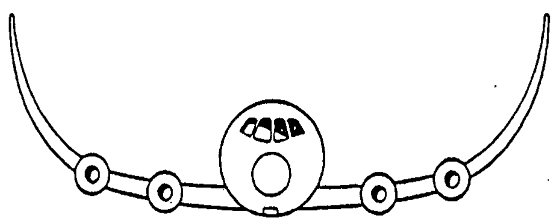


Figure 5. Aircraft with strain of 1·6 per cent in wing spar booms.
$\left(\text{Bend radius of beam} = \frac{\text{thickness}}{2 \times \text{strain}}\right)$

or have the proper clearances, when the load is on.† A knowledge of the E of the material being used is the first thing we need to know in making these calculations. Secondly, although the lay-

* S. B. Hamilton, *History of Technology*, vol. 4, chapter 15.

† I once did a design study of a plastic railway carriage for British Rail. One of the troubles was that, if the doors fitted properly when the carriage was empty, they would neither open nor close when the carriage was full of passengers in the rush hour.

man might suppose, as the early engineers seem to have done, that the stiffness of all common structural materials were very similar ('Well, it's stiff, isn't it, you can't *see* any deflections'), this is in fact very far from being the case and we not only need to know the Es of various materials such as wood and steel in order to calculate their deflections, but we must also arrange that the deflections of differing materials in a structure are compatible and that they share the load in the way we want them to.

Since, if we divide stress by a ratio – that is by a number without dimensions* – we must still have a stress, Young's modulus is therefore a stress in pounds per square inch, or what you will. It is that stress which would in theory double the length of a specimen, if it did not break first. One can also regard it as the stress to produce 100 per cent strain. As it will easily be imagined, the actual figure is likely to be a high one, usually at least a hundred times larger than the breaking stress of the material, because, as we have said, materials are apt to fracture in the ordinary way at 1 per cent elastic strain or less. The Young's modulus of steel, for example, is about 30,000,000 pounds per square inch. As we have also said, E varies very much according to the kind of chemical substance we are dealing with. A few typical figures are shown on page 42.

Thus the whole range of solids vary in E by about 200,000 to 1. Even substances which we normally think of as 'rigid' vary by about 1000 to 1, which is still an enormous range. E is very low in rubber because rubber is made of long molecular chains which are flexible and in the resting material they are generally much bent, kinked and convoluted, like a heap of bits of string such as one finds in a drawer in the hall at home. When rubber is stretched, the bent chains are straightened and, as one can easily see, the force needed to do so is very much less than that which is needed to stretch an arrangement of strings which were initially straight. Nothing of this kind happens in a normal crystal where one is pulling directly on the interatomic bonds and the only reason for the large variations in Young's modulus is that the chemical bonds themselves vary a great deal in stiffness. So with crystals, although the general shape of the interatomic force curves is

* i.e. by a strain.

Approximate Young's moduli of various substances

	E Pounds per square inch	E MN/m^2
Rubber	$0{\cdot}001 \times 10^6$ (i.e. 1,000)	7
Unreinforced plastics	$0{\cdot}2 \times 10^6$	1,400
Organic molecular crystal, phthalocyanine, a blue pigment	$0{\cdot}2 \times 10^6$	1,400
Wood (about)	$2{\cdot}0 \times 10^6$	14,000
Concrete	$2{\cdot}5 \times 10^6$	17,000
Bone	$3{\cdot}0 \times 10^6$	21,000
Magnesium metal	$6{\cdot}0 \times 10^6$	42,000
Ordinary glasses	$10{\cdot}0 \times 10^6$	70,000
Aluminium	$10{\cdot}5 \times 10^6$	73,000
Steel	$30{\cdot}0 \times 10^6$	210,000
Aluminium oxide (sapphire)	$60{\cdot}0 \times 10^6$	420,000
Diamond	$170{\cdot}0 \times 10^6$	1,200,000

Note. Because the interatomic force curve (Figure 3) passes smoothly through the point of zero stress and strain the true E of a material is always the same in compression as it is in tension at all normal strains. If this were not so then the mathematics of elasticity would be even more complicated than they are. In practice, however, materials such as cast iron and cement, which contain quite gross internal cracks, may sometimes show an E which is lower in tension than it is in compression. This is simply because the cracks gape under tension and 'come up solid' under compression.

similar, the slope of the straight part of the curves varies greatly according to the bond energy and other chemical conditions.

The figure for the E of phthalocyanine tells us at once why a great many solid chemical compounds are not candidates for the status of structural materials. Generally speaking we want a structure to be as rigid as possible: bridges and buildings sway quite enough as it is and there are excellent reasons for making other things rigid as well. Any structure made from a material with a stiffness as low as phthalocyanine would be far too floppy. Steel is about the stiffest reasonably cheap material, which is one of the reasons why it is used so widely. As much as anything it is the relatively low stiffness of plastics, even when 'reinforced', which restricts their use for large objects.

Strength

Next to 'heat-proof' I suppose that 'unbreakable' is one of the most useful words in advertising. Although most of us know that advertising is not an entirely objective profession, somehow or other the message sinks in so that one still meets people who really believe that there are unbreakable objects or, if there aren't, then there ought to be. Since there is always some force which will tear the atoms apart in a solid (since the chemical bonds have a finite energy or, in other words, they are only so strong) nothing is unbreakable. You have only to get hold of the thing firmly and pull hard enough and it will break. The only question is 'how soon?' There is however a very large variation between the strengths of various materials.

Lest there be any possible, probable, shadow of doubt, strength is not, repeat not, the same thing as stiffness. Stiffness, Young's modulus or E, *is concerned with how stiff, flexible, springy or floppy a material is. Strength is the force or stress needed to break a thing. A biscuit is stiff but weak, steel is stiff and strong, nylon is flexible* (*low* E) *and strong, raspberry jelly is flexible* (*low* E) *and weak. The two properties together describe a solid about as well as you can reasonably expect two figures to do.*

It is easiest to think about strength in terms of tensile strength. This is the stress needed to pull a material asunder by breaking all the bonds between the atoms along the line of fracture. One can perhaps most conveniently think of it as the stress required to break a bar by pulling it along its axis like a rope. A very strong steel may withstand a tensile stress of 450,000 pounds (200 tons) per square inch (3,000 MN/m^2), while ordinary brick or cement may perhaps withstand 600 or 800 p.s.i. or only 4 or 5 MN/m^2.* The strength of commonly used engineering materials thus varies over a range of about a thousand to one†. The tensile strengths of some common materials are given in the table.

* 'Daddy, why can't you make boilers out of cement?'

† For the moment we may be content to say that breaking stress is that stress at which things break. However, let us beware of a trap or incipient muddle. If a bar of 10 square inches cross section breaks under a tensile load of a 100 tons then its breaking load is a 100 tons, but its breaking stress

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(54) **Gewebekonstrukt für die Transplantationschirurgie**

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Construction cellulaire pour la chirurgie de transplantation

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(73) Patentinhaber: **Weitzel-Kage, Doris
12207 Berlin (DE)**

(72) Erfinder:
- **Weitzel-Kage, Doris
12207 Berlin (DE)**
- **Salomon, Andreas
13503 Berlin (DE)**

(74) Vertreter: **Jungblut, Bernhard Jakob, Dr. et al
Albrecht, Lüke & Jungblut,
Patentanwälte,
Gelfertstrasse 56
14195 Berlin (DE)**

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nung ist die Längendifferenz dividiert durch die ursprüngliche Länge. Mit einem erfindungsgemäßen Konstrukt wird, mit anderen Worten ausgedrückt, neue Haut mit ihrer Schichtstruktur nicht in vitro sondern erst in vivo, also nach Aufbringen des Gewebekonstrukts, gebildet.

[0008] Mit der Erfindung wird eine überraschend hohe Sicherheit hinsichtlich des Anwachsens des Gewebekonstrukts in einer Fehlstelle erreicht. Nachträgliche Maßnahmen, insbesondere erneute Transplantationen sind in praktisch allen Fällen entbehrlich. Selbstverständlich kann aus Gründen der Sicherheit ein Wiederholungsgang durchgeführt werden. Die mit dem Gewebekonstrukt neu gebildete Oberflache, insbesondere Haut, wird gut durchblutet und bildet kein störendes Narbengewebe. Es wird eine kosmetisch in beachtlichem Maße zufriedenstellende Hautneubildung erreicht. Ohne an eine Theorie gebunden zu sein, wird vermutet, daß aufgrund nicht vorhandener Kontaktinhibierung das Gewebekonstrukt besonders gut in eventuelle Lücken hineinwachsen kann. Durch die elastischen Eigenschaften des Trägermaterials werden eventuelle Lücken zudem recht klein gehalten, da sich ein erfindungsgemäßes Gewebekonstrukt mit sogenanntem saugenden Kontakt einbringen läßt.

[0009] Je nach Anwendung ist es bevorzugt, wenn die Körperoberflächenzellen ausgewählt sind aus der Gruppe bestehend aus "Keratinozyten und Epithelzellen des Respirationstrakts, der Mundschleimhaut, der Cornea oder innerer Körperoberflächen wie Harnblase, Gallenblase und dergleichen". Falls das Gewebekonstrukt zum Ersatz zerstörter Haut verwendet werden soll, so ist der Einsatz von Keratinozyten bevorzugt. Es kann auch mit Gewebekonstrukten enthaltend Fettzellen oder Fibroblasten gearbeitet werden.

[0010] Bei der Regeneration von Haut können erfindungsgemäße Gewebekonstrukte mit Fettzellen und/oder Fibroblasten und/oder Keratinozyten in dieser Reihenfolge in zeitlichem Abstand (1 bis 5 Tage) und in der angegebenen Reihenfolge aufgebracht werden. Es ist aber im Rahmen der Erfindung auch möglich, ein Multilayersandwich enthaltend die Schichtfolge (von der Wunde aus gesehen) "Fettzellen, Substrat, Fibroblasten, Substrat, Keratinozyten, Substrat" herzustellen und aufzubringen. In letzteren Falle werden die Substratschichten nach der Applikation subsequent von der Wundenseite her aufgelöst aufgrund der in der Wunde vorliegenden Kollagenasen. Mit dem Einsatz von Fibroblasten wird die Ausbildung von neuem Bindegewebe gefördert. In beiden Alternativen dieses Ausführungsbeispiels können die Fettzellen oder die Fibroblasten weggelassen werden.

[0011] Hinsichtlich der Zelldichte der Körperoberflächenzellen ist es bevorzugt, daß zumindest 20%, vorzugsweise zumindest 50%, höchstvorzugsweise zumindest 70%, besser 80 oder 90 bis 100%, der Körperoberflächenzellen nicht kontaktinhibiert sind. Je höher der Anteil nicht kontaktinhibierter Körperoberflächenzellen ist, um so besser gelingt das Anwachsen des Gewebekonstrukts in einer Fehlstelle.

[0012] Hinsichtlich der mechanischen Eigenschaften ist es bevorzugt, wenn das Trägermaterial ein Elastizitätsmodul von weniger als 1 N/mm^2, vorzugsweise von weniger als 0,5 N/mm^2, höchstvorzugsweise im Bereich von 0,05 bis 0,15 N/mm^2 aufweist. Je elastischer das Trägermaterial ist, um so leichter läßt sich das Gewebekonstrukt in einer (dreidimensional) komplexen Fehlstelle vollständig zum Anliegen bringen. Die Bruchdehnung sollte möglichst hoch sein und zumindest oberhalb von 20%, besser oberhalb von 50% liegen. Die Bruchdehnung ist definiert als die Dehnung, bei welcher Bruch erfolgt. Es versteht sich, daß das Elastizitätsmodul nicht so klein sein darf, daß unter dem Eigengewicht des Gewebekonstrukts die Bruchdehnung erreicht wird. Die angegebenen Werte beziehen sich auf 18°C und Luftumgebung. Nach dem Einbringen eines erfindungsgemaßen Konstrukts in eine Wunde ändern sich die Materialeigenschaften. Typischerweise hat das Substrat eine Dicke von 0,1 bis 5 mm, vorzugsweise von 0,3 bis 2 mm, höchstvorzugsweise von 0,3 bis 1 mm.

[0013] In optischer Hinsicht ist es bevorzugt, wenn das Substrat transparent und optisch klar ist. Dann kann der Kultivierungsfortschritt ohne weiteres mit einem Inverslichtmikroskop beobachtet werden. Dies ist von durchaus wesentlicher praktischer Bedeutung, da dann die Kultivierung leicht überwacht und bei Erreichen einer gewünschten Zelldichte bzw. vor Erreichen einer unerwünscht hohen Kontaktinhibierung abgebrochen werden kann.

[0014] Ein allen mechanischen Anforderungen genügendes Gewebekonstrukt wird erhalten, wenn das Trägermaterial aus mittels eines Elastizität vermittelnden Vernetzungsmittels vernetzter Gelatine besteht. Gelatine ist ein Abbauprodukt des Collagens. Die Gelatine kann aus Collagenen verschiedener Typen, also aus Collagen I bis IV gewonnen werden. Das Collagen kann menschlicher oder tierischer Herkunft sein. Gut geeignet ist eine Gelatine aus Schweinehaut mit einer Bloom-Stärke von 100 bis 400, beispielsweise 300. Als Vernetzungsmittel kommen alle denkbaren Stoffe in Frage, sofern sie einerseits physiologisch unbedenklich und andererseits in ausreichendem Maße elastizitätsvermittelnd sind. In chemischer Hinsicht ist das Vernetzungsmittel bevorzugt eine organische Verbindung der Formel R1-X-R2 ist, wobei R1 und R2 gleich oder verschieden sein können und Gruppen darstellen, welche mit Aminogruppen und/oder Carboxylgruppen, ggf. auch mit Sulfhydrylgruppen, umsetzbar sind und wobei X C2-C20-Alkyl ggf. mit einer Elastizität vermittelnden funktionellen Gruppe ist. Für R1 und R2 sind beispielsweise zu nennen: -CHO, Imidoester eines C1 bis C5 Alkanols, N-Hydroxysuccinimidyester, Maleinimid, α-Haloacetal, Pyridyldisulfid. Als Beispiel einer Elastizität vermittelnden Gruppe ist Carbamat zu nennen. Bevorzugt sind homobifunktionelle Vernetzungsmittel, insbesondere 1,5-Pentandial.

[0015] Die Herstellung des Trägermaterials kann ausgehen von einer 7 bis 25%-igen (w/v), vorzugsweise einer 10 bis 20%-igen, höchstvorzugsweise einer 10 bis 14%-igen wäßrigen Gelatinelösung. Bei ca. 20% wird eine zwar ge-